1. When you receive your primers:
    - Resuspend primers in nm (found on the side of tube) x10µl ddH2O
        - For example, 26.6nm, resuspend in 266ul H20. This is your 10x stock and then dilute 10-fold more for your working stock.
    - A working stock = 1 part above solution : 9 part ddH2O
        - For example 10ul of your 10x stock + 90ul of ddH20
    - The working stock can be used for PCR

2. Find your template and check the concentration (ng/ul)

3. Thaw your dNTP's, PCR buffer, primers, and template on ice

4. Prepare your PCR mix. Calculate how much water and each of the ingredients needs to be added. A typical reaction setup is shown below. Add water first, then add the top 5 ingredients (through template). DO NOT add the polymerase until you are almost ready to put the samples in the PCR machine. Polymerase is kept at -20°C until needed and even then is kept in a block cooler.

COMPONENT	For a 50 ul reaction
5X Q5 Reaction Buffer	10 ul
10 mM dNTPs	1 ul
10 µM Forward Primer	1 ul
10 µM Reverse Primer	1 ul
Template DNA	0.5 ul
Q5 High-Fidelity DNA Polymerase	0.5 ul
5X Q5 High GC Enhancer (optional)	*(ignore, will generally not add)*
Nuclease-Free Water	36 ul

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25–35 Cycles	98°C	5–10 seconds
	*50–72°C	10–30 seconds
	72°C	20–30 seconds/kb
Final Extension	72°C	2 minutes
Hold	4–10°C	

Note: Our default reaction will have an annealing temperature of 55°C for 20 seconds and extension at 72°C for 25sec/kb.

5. Setup the PCR thermocycler and once it has the correct protocol, add polymerase.
6. Quickly spin down your PCR tubes in the minicentrifuge (1-2 second spin)
7. Place tubes in thermocycler and start PCR.
8. Pour an agarose gel while you are waiting.
    a. Weigh agarose, generally will pour a 1% gel = 0.4 g Agarose in 40 ml TAE.
    b. Place agarose in a small beaker along with appropriate volume TAE
    c. Microwave slowly until dissolved. Swirl every 10-15 seconds to avoid boiling over